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Glutamate dysfunction in people with prodromal symptoms of psychosis: relationship to gray matter volume

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Background: The glutamate model of schizophrenia proposes that altered glutamatergic neurotransmission is fundamental to the development of the disorder. In addition, its potential to mediate neurotoxicity raises the possibility that glutamate dysfunction could underlie neuroanatomical changes in schizophrenia. Here we determine whether changes in brain glutamate are present in subjects at ultra high risk of developing psychosis, and whether these changes are related to reductions in cortical gray matter volume.

Methods: Twenty-seven individuals with an At Risk Mental State (ARMS) and a group of 27 healthy volunteers underwent proton magnetic resonance spectroscopy and volumetric proton magnetic resonance imaging using a 3 Tesla scanner. Glutamate and glutamine levels were measured in anterior cingulate, left hippocampus and left thalamus. These measures were then related to cortical gray matter volume.

Results: ARMS subjects had significantly lower levels of glutamate than controls in the thalamus ($p<0.05$), but higher glutamine in the anterior cingulate ($p<0.05$). Within the ARMS group, the level of thalamic glutamate was directly correlated with gray matter volume in the medial temporal cortex and insula ($p<0.01$).

Conclusions: This study provides the first evidence that brain glutamate function is perturbed in people with prodromal signs of schizophrenia, and that glutamatergic dysfunction is associated with a reduction in gray matter volume in brain regions thought to be critical to the pathogenesis of the disorder. These findings support the hypothesis that drugs affecting the glutamate system may be of benefit in the early stages of psychotic illness.

Introduction

Altered brain glutamatergic transmission is thought to be one of the primary neurochemical abnormalities in schizophrenia (1-3). Uncompetitive NMDA receptor antagonists such as phencyclidine (PCP) and ketamine consistently induce effects resembling the positive and negative symptoms of schizophrenia in humans. These drugs have been shown to cause an increase in glutamate release in prefrontal cortex in animal studies (4,5), and to be associated with toxic changes in cortical neurons (6,7). These effects are hypothesised to occur through preferential blockade of NMDA receptors expressed on GABAergic interneurons, leading to a disinhibition of glutamatergic neurons projecting to the cerebral cortex (6). The thalamus may be the primary site where this block occurs: direct injection of NMDA receptor antagonists into cortical brain regions does not cause glutamate (Glu) release (4), or neuronal toxicity (7), whereas injection into thalamus induces an identical pattern of toxic change in the cortex to that seen with systemic administration (7). Moreover, injection of GABA agonists into thalamus prevents the cortical changes induced by systemic administration of NMDA receptor anagonists (7).

Neuroimaging studies in patients with schizophrenia have provided some evidence of NMDA receptor dysfunction, and of a disinhibition of Glu release. One single photon emission computed tomography (SPECT) study of medication-free patients found that they had reduced hippocampal NMDA receptor binding relative to whole cortex, and that this was partially reversed in patients on antipsychotic medication (8). Another study, using proton magnetic resonance spectroscopy (1H-MRS) found that unmedicated first episode patients had elevated glutamine (Gln) in anterior cingulate (9). Patients with first episode and chronic schizophrenia have also been found to

have elevated Gln levels in the thalamus (9,10). Although 1H-MRS cannot distinguish between metabolic and neurotransmitter Glu, there is evidence that 80-100% of Glu in the brain is rapidly cycled to Gln through its release as a neurotransmitter (11). As Gln is generated from Glu in astrocytes after the release of Glu from the synapse, the level of Gln measured with 1H-MRS has been suggested as a marker of the degree of Glu release in a given region (9). In keeping with this hypothesis, ketamine led to increased anterior cingulate levels of Gln in healthy controls, measured using 1H-MRS (12).

Structural MRI studies of schizophrenia show robust reductions in regional gray matter volume (13). Longitudinal reductions in gray matter volume appear to occur after the first episode of schizophrenia, and the degree of gray matter loss in the early phase of the illness may predict subsequent clinical outcome (14,15). The underlying basis of these volumetric changes is unknown. The role of elevated synaptic Glu release is of particular interest in this context, as its potential for excitotoxicity and modulation of plasticity through effects on NMDA receptors could give rise to changes in gray matter volume (16,17).

The first episode of schizophrenia is usually preceded by a prodromal phase characterized by attenuated psychotic symptoms in the context of a marked decline in global function. This At Risk Mental State (ARMS) is associated with a greatly increased risk of developing psychosis, with a transition rate of around 35% within 24 months (18,19). Volumetric MRI studies of the ARMS indicate that it is associated with reductions in regional gray matter volume that are qualitatively similar but less severe than are evident in schizophrenia (20-22).

In this study we used 1H-MRS and volumetric MRI to address the question of whether glutamatergic abnormalities are present in ARMS subjects and, if so, how they are related to the alterations in gray matter volume that are evident in this group. We tested the following hypotheses: 1) Regional Glu and Gln levels would be significantly different in ARMS subjects and controls 2) ARMS subjects would have reduced regional gray matter volume relative to controls 3) The alteration in Glu and Gln levels in the ARMS group would be correlated with relative reductions in gray matter volume.

Methods

Ethics and power calculation

Ethical approval for this study was obtained from the South London and Maudsley NHS Trust Ethics Committee. Sample sizes were estimated from a pilot study of 4 healthy controls with test-retest (mean Glu + Gln=17.02 institutional units, (iu), SD=3.63). We used G*Power statistical software (23), to calculate that 26 subjects in each group would be required to detect a 20% difference in anterior cingulate Glu + Gln with a power of 0.9 (alpha=0.05, two tailed).

Informed consent

ARMS subjects were recruited from OASIS (Outreach and Support in South London), part of the South London and Maudsley NHS Trust. The study was explained to them and they were given a written description of the study. Controls were recruited from the same geographical area through advertisements that outlined the study. All

subjects who expressed interest in participation were offered a face-to-face interview, where the full details of the study, including its possible risks and benefits were explained. They were then invited to give written consent to take part, and told that they were free to withdraw from the study at any time without giving a reason.

Sample

We compared twenty-seven individuals meeting PACE criteria for the ARMS (24), and twenty-seven healthy volunteers. Controls had no personal history of psychiatric symptoms, psychotropic medication or medical illness, and no family history of psychiatric illness. For both groups, exclusion criteria included history of severe head injury (loss of consciousness for over 5 minutes), drug or alcohol dependence, metallic implants, and pregnancy.

Pre-scan interview

Prior to scanning, all subjects were interviewed about their family and personal psychiatric history, current and past medication use and current and past use of alcohol, tobacco and illicit drugs. Symptomatology in both groups was assessed using the Comprehensive Assessment of At-Risk Mental State (CAARMS) (24), The Positive and Negative Symptom Scale (PANSS) (25), and the Hamilton Depression and Anxiety Scales (HAM-D and HAM-A) (26,27).

Selection of regions of interest

We selected the anterior cingulate cortex and the left thalamus as regions of interest (ROIs) for spectroscopic analysis, on the basis of previous findings of altered Gln levels in anterior cingulate cortex in patients with schizophrenia (9), and of altered Gln levels in the left thalamus in schizophrenia (9,10,28). The thalamus is of additional interest, as NMDA receptor dysfunction at this site putatively underlies the elevation of cortical glutamatergic transmission that is thought to occur in schizophrenia (6,7). The third ROI chosen was the left hippocampus, the hippocampus having been implicated as a site of glutamatergic abnormality (8,29,30), as well as a commonly reported site of volume loss in schizophrenia (31).

Volumetric MRI protocol

All subjects underwent volumetric MRI and 1H-MRS scanning. Scanning took place on a General Electric (Milwaukee, USA) 3 Tesla HDx Magnetic Resonance system. After positioning the subject in the scanner with a foam rest under their knees, an initial localizer scan was performed to measure the interhemispheric angle and the AC-PC line (the line passing through the upper part of the anterior commisure and the lower part of the posterior commisure – approximated from the anterior and posterior corpus callosum).

Structural images were acquired using an axial 2D T_2 weighted Fast Spin Echo scan and an axial fast FLAIR scan (total scan time 5 minutes), both prescribed parallel to the AC-PC line. These were followed by a whole brain 3D coronal IR-SPGR (inversion recovery prepared spoiled gradient echo) scan, prescribed from the midline

sagittal localizer, giving isotropic 1.1mm resolution in a scan time of approximately 6 minutes (TE = 2.82ms; TR = 6.96ms; TI = 450ms; flip angle = 20º). The IR-SPGR scans were used for localization of the spectroscopy ROIs, and were subsequently segmented into gray matter, white matter and CSF using SPM2 to allow correction of the spectroscopy results for partial volume CSF contamination. IR-SPGR scans were also segmented with SPM5 for voxel based morphometry, with non-parametric statistical testing of group differences in gray matter volume being performed using BAMM (Brain Activation and Morphological Mapping; http://www-bmu.psychiatry.cam.ac.uk/BAMM/index.html).

1H-MRS protocol

All subjects were scanned using the scanner's body coil for transmit, and the manufacturer's standard 8-channel coil for receive. 1H-MRS spectra (PRESS - Point RESolved Spectroscopy - TE=30ms, TR=3000ms, 96 averages) were acquired in the anterior cingulate, left hippocampus and left thalamus. We employed the standard GE PROBE (proton brain exam) sequence, which uses a standardized chemically selective suppression (CHESS) water suppression routine. For each metabolite spectrum, unsuppressed water reference spectra (16 averages) were also acquired as part of the standard acquisition. Shimming and water suppression were optimized, with auto-prescan being performed twice prior to each scan. The anterior cingulate ROI was prescribed from the midline sagittal localiser, and the centre of the 20mm x 20mm x 20mm ROI was placed 13mm above the anterior section of the Genu of Corpus Callosum at 90^{o} to the AC-PC line. A 20mm x 20mm x 15mm (right-left, anterior-posterior, superior-inferior) left hippocampal ROI was prescribed from a coronal SPGR image. A 15mm x 20mm x 20mm (right-left, anterior-posterior,

superior-inferior) left thalamus ROI was defined at the point in the coronal slices where the thalamus was widest, using sagittal and coronal localisers to ensure that the ROI was clear of CSF contamination. After the subject left the scanner, each scanning session concluded with the collection of a PRESS spectrum from a phantom containing standard concentrations of brain metabolites to allow monitoring of scanner drift or step-changes with scanner software updates over the period of data acquisition for this study.

1H-MRS quantification

All spectra were analysed using LCModel version 6.1-4F (32). The raw spectral data were read into LCMgui, the graphical user interface for LCModel, which automatically combined the data from the 8-channel coil with a weighted coherent average over the 8 receive channels using the intensity of the first point of the FID of the unsuppressed water reference from each coil. A standard basis set of 16 metabolites (L-alanine, aspartate, creatine, phosphocreatine, GABA, glucose, glutamine, glutamate, glycerophosphocholine, glycine, myo-inositol, L-lactate, N-acetylaspartate, N-acetylaspartylglutamate, phosphocholine, taurine), included as part of LCModel, and acquired with the same field strength (3T), localization sequence (PRESS) and echo time (30ms) as our study was used. Model metabolites and concentrations employed in the basis set are fully detailed in the LCModel manual (http://s-provencher.com/pages/lcm-manual.shtml).

Poorly fitted metabolite peaks (Cramer-Rao minimum variance bounds of more than 20% reported by LCModel) were excluded from further analysis. Water-scaled Glu, Gln and NAA values were corrected for the CSF content of the ROI using the formula

M_{corr}=M/(1-C) where M is the uncorrected metabolite value and C is the fractional CSF content of the ROI. We determined the CSF content of each ROI for each subject by extracting the size and location of the ROI from the spectra file headers, and using an in-house program to calculate the percentage grey, white and CSF content using the segmented IR-SPGR images.

Statistics

All statistical analyses were performed using SPSS version 16.0 (SPSS inc. Chicago, Illinois, USA). A GLM multivariate ANOVA was performed with diagnostic group as a factor and levels of Glu and NAA in each region as dependent variables. Due to the small number of subjects with well-fitted Gln peaks, group differences in Gln levels were entered into the GLM in a subsequent step. The effect of drug use and of demographic differences between groups on significantly different metabolite measures was studied using linear regression (stepwise).

MRI data analysis

Segmentation was performed using Statistical Parametric Mapping software (SPM5, Wellcome Department of Imaging Neurosciences, University College London, UK). Gray matter probability images were then "modulated" (to compensate for the effect of spatial normalisation) by multiplying each voxel value by its relative volume before and after warping. The segmented images were then smoothed with an 8mm x 8mm x 8mm Gaussian kernel to reduce noise, and also allow for the effects of small residual mis-registrations. Given that structural brain changes are likely to extend over a number of contiguous voxels, test statistics incorporating spatial information such as 3D cluster mass (the sum of suprathreshold voxel statistics) are generally

more powerful than other possible test statistics, which are informed only by data at a single voxel (33). As no parametric distribution is known for cluster mass, permutation testing was used to assess statistical significance.

Between-group differences in gray matter volume, and correlations of gray matter volume difference with significant CSF-corrected metabolite differences were analysed by fitting an analysis of covariance (ANCOVA) model at each intracerebral voxel in standard space, covarying for total gray matter, using the BAMM package. A relatively lenient p-value (p=0.05) was initially set to detect voxels putatively demonstrating differences between groups; spatial clusters of such voxels were then searched for and the "mass" of each cluster found (the sum of suprathreshold voxel statistics it comprises) was tested for significance. Permutation based testing, implemented in the BAMM package, was used to assess statistical significance at both the voxel and cluster levels (33). At the cluster level, rather than set a single a priori p-value below which findings are regarded as significant, the number of clusters which would be expected by chance alone for a range of p-values was calculated. The statistical threshold for cluster significance for each analysis was then set such that the expected number of false positive clusters by chance alone would be less than one (33).

Results

Subject demographics

Control and ARMS individuals did not differ in age, sex, ethnicity, social class or IQ (measured using NART), but controls had a significantly higher level of education than ARMS individuals (Table 1). Eight ARMS individuals but none of the controls

had previously taken antidepressant or antipsychotic medication and five of these were still taking medication (four had taken quetiapine, but only one was taking it at the time of the first scan, one was taking aripiprazole, two were taking citalopram and one was taking sertraline). There were no significant group differences in substance or alcohol use, but the ARMS subjects were more likely to have used tobacco.

Clinical measures

ARMS subjects had significantly higher levels of prodromal, psychotic, anxiety and depressive symptoms than controls, as measured using the CAARMS, PANSS, HAM-A and HAM-D (see table 2).

1H-MRS measures

There was no evidence from serial water-scaled phantom measurements of scanner drift or step changes in estimated metabolite values during the acqusition period for this study. 1H-MRS spectra quality were good in left thalamus and in anterior cingulate, with a mean(SD) signal to noise ratio reported by LCModel of 19(4) and 19(6) respectively, and of reasonable quality in left hippocampus with a mean(SD) signal to noise ratio of 12(3). Linewidths reported by LCModel followed a similar pattern with mean(SD) of 5.3(1.8) Hz in anterior cingulate, 6.6(1.4) Hz in left thalamus and 8.9(3.1) Hz in left hippocampus. There were no significant differences in spectral quality between control and ARMS subjects.

Glu (water-scaled, CSF corrected) and NAA in left thalamus were both significantly lower in ARMS subjects than controls ($F_{(1,43)}$=7.545, p=0.008; $F_{(1,43)}$=7.450, p=0.009). There was also a significant elevation of Gln levels in the anterior cingulate in ARMS subjects compared to controls ($F_{(1,17)}$=6.998, p=0.017). Linear regression

revealed that these results were best explained by diagnostic status alone: previous exposure to psychotropic medication, educational level, and current level of tobacco, alcohol or cannabis use did not have any additional predictive value for Glu, Gln, or NAA levels. Furthermore, the results remained significant whether or not CSF correction was employed, indicating they were not an artefact of the CSF correction.

In keeping with the hypothesis that NAA is a marker for pyramidal cell integrity, Glu correlated positively with NAA in all three ROIs. This relationship was stronger in anterior cingulate ($r=0.703$, $n=53$, $p<0.001$) and left hippocampus ($r=0.550$, $n=51$, $p<0.001$) than in left thalamus ($r=0.331$, $n=52$, $p<0.05$).

Other measured metabolites, for which we had no a priori hypotheses, have been summarized in Table 3. Thalamic creatine was reduced in ARMS subjects, although this finding does not survive correction for multiple comparisons.

Group differences in regional gray matter volume

ARMS subjects had less gray matter volume than controls in the orbitofrontal cortex bilaterally, extending into the adjacent ventral anterior cingulate cortex, but relatively more gray matter volume in left cerebellum and left occipital cortex (cluster threshold $p<0.007$; see figure 1).

Relationship between 1H-MRS measures and gray matter volume

In ARMS subjects, levels of Glu in left thalamus were directly correlated with gray matter volume in the left prefrontal cortex, insula, cingulate, superior temporal gyrus and temporal pole, as well as bilaterally in the cerebellum and lingual gyrus (cluster

p<0.005; Figure 2). In these regions, the lower the thalamic Glu level, the smaller the volume of gray matter. Conversely, there was an inverse correlation between left thalamic Glu and the volume of the dorsal anterior cingulate extending to the posterior cingulate gyrus (cluster p<0.005; Figure 2). Thalamic NAA did not correlate with gray matter in any brain region in ARMS subjects.

Anterior cingulate Gln in the ARMS subjects was inversely correlated with gray matter in left cerebellum, and directly correlated with gray matter volume in the posterior cingulate gyrus (threshold p<0.004). This region was contiguous with, but did not significantly overlap, the portion of the cingulate gyrus where there was a negative correlation with thalamic Glu levels.

We did not have any a priori hypotheses about correlations between Glu or Gln and gray matter volume in healthy controls. A post-hoc analysis revealed there were no significant correlations between thalamic Glu or NAA and grey matter in healthy controls. However, there was an inverse correlation between anterior cingulate Gln levels and grey matter volume in medial frontal and orbitofrontal cortex, and a positive correlation with grey matter volume in right temporal cortex.

Discussion

This is the first 1H-MRS study to examine Glu function in subjects experiencing prodromal symptoms of schizophrenia, and the first study to examine the relationship between Glu levels in these subjects and gray matter volume. The findings are not attributable to effects of antipsychotic medication, as most of the ARMS subjects

were medication naive, and inclusion of medication as a factor in the analysis had no effect on the results.

There are some potential limitations to this work that should be considered. 1H-MRS is a difficult imaging method to apply, with a potential for erroneous results if poor quality spectra are included in the analysis (34). In the present study this led to us having to exclude a large number of Gln estimates, particularly in the left hippocampal and thalamic ROIs. The reason for the difficulties in measuring Gln probably relate to the fact that this study was performed using a 3T scanner, whereas groups studying Gln as a separate peak have generally employed 4T scanners (9,10,12). The fact that we could only obtain reliable measures of Gln in anterior cingulate is likely to be a result of the better quality (lower linewidth) spectra obtained from this region. Poor fitting of Gln could also, theoretically, have led to overestimation of the overlying NAA peaks (35).

Differences in tissue relaxation times between patients and controls, as previously reported in patients with schizophrenia (36), could lead to differences in estimation of water-scaled metabolite concentrations. We investigated this possibility by extrapolating the previously reported values to 3T and found that, if differences in tissue water relaxation were as marked in ARMS subjects as in patients with schizophrenia, metabolite estimation could deviate by a maximum error of 3%.

The two groups we studied were not matched for tobacco use, with higher rates of use in the ARMS subjects. This is consistent with evidence that patients with schizophrenia use more tobacco than patients with other psychiatric disorders and

controls (37), and that adolescents who later develop schizophrenia have higher rates of smoking before the onset of illness (38). However, history of tobacco use was not found to be a predictive factor for either thalamic Glu or NAA or anterior cingulate Gln.

We found reduced regional gray matter volume in ARMS subjects compared to healthy controls. This is consistent with the results of previous MRI studies (20-22), which indicate that reductions in regional gray matter volume are evident in people with prodromal symptoms of psychosis. As in these studies, the location of the volumetric findings in the present study correspond to sites of reduced gray matter volume in patients with schizophrenia (39). The region of increased gray matter in ARMS subjects compared to controls including occipital cortex was in keeping with an earlier study with a different cohort of patients, reporting that ARMS subjects who underwent transition to psychosis had increased grey matter in occipital cortex compared to those who did not (21).

Thalamic Glu and NAA levels in the left thalamus were significantly reduced in the ARMS group. 1H-MRS studies in schizophrenia have generally reported elevated thalamic Gln levels, and unchanged Glu levels, although these have involved patients with established psychosis rather than subjects with prodromal symptoms (9,10,28). The reason for the differences between the results of our study and previous studies is not clear. The method we used to measure Glu and Gln (LCModel) was different from that used by Theberge *et al.* (9,10,28), who used in-house software (Fitman and LHRI Analysis Suite, Theberge - personal communication 25th March 2008). There are differences between these two methods of quantifying Glu and Gln: for example, the

LCModel uses non-physical model components to perform baseline fitting (spline fit). There were also differences between these studies in 1H-MRS acquisition (PRESS vs. STEAM), the method for selection of good quality spectra, and in scanner model and field strength (3T vs. 4T). Finally, the studies examined quite different groups: we examined individuals at high risk of psychosis, whereas the previous studies investigated patients with schizophrenia. Interestingly, a previous study found a correlation between reductions in NAA in left thalamus in patients with first episode psychosis and the length of the preceding prodromal phase (40).

We found that Gln levels in the anterior cingulate cortex were elevated in the ARMS group relative to controls. This finding is in keeping with previous reports of increased cingulate Gln levels in unmedicated patients with first-episode schizophrenia (9), and in the adolescent relatives of patients with schizophrenia (41).

One of the most striking findings was the relationship between thalamic Glu levels in the ARMS group and regional gray matter volume. We found that the lower the Glu levels, the smaller the gray matter volume in the medial temporal, lateral temporal, inferior frontal, insula and cingulate cortex, as well as in the cerebellum. This set of areas corresponds closely to the sites of the most robust reductions in volume in MRI studies of schizophrenia (39). The finding is also consistent with data from a recent 1H-MRS and MRI study in schizophrenia, which found that a longitudinal reduction in thalamic Gln levels was correlated with a progressive reduction in parietal and temporal cortex gray matter volume (28). The correlation between thalamic Glu levels and reductions in gray matter volume we observed raises the possibility that changes in Glu function might contribute to the structural findings, possibly through

disinhibition of thalamocortical pyramidal cells (17). Unexpectedly, the reduction in thalamic Glu in the ARMS group was also associated with relatively *increased* gray matter volume in the dorsal and posterior cingulate cortex. It has been suggested that increases in gray matter volume might occur in the very early stages of apoptosis (42), so it possible that this might be a relatively early effect of disinhibition of thalamocortical glutamatergic projections, with a reduction in cortical volume occurring at a later stage. We are currently collecting longitudinal 1H-MRS data in this sample, which may clarify whether the relationship between thalamic Glu and cortical gray matter volume changes over time.

Conclusions

This study suggests that cortical and thalamic Glu function are perturbed in people at ultra high risk of developing psychosis, and that thalamic reductions in Glu are related to alterations in cortical gray matter volume in this group. Future work will determine whether thalamic Glu reductions are related to risk of transition to psychosis, and whether pharmacological modulation of the Glu system can reduce this risk.

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The authors report no biomedical financial interests or potential conflicts of interest.

Table 1: subject demographics

	ARMS	Control	Significance
Age mean(SD)	25(5)	25(4)	P=0.883
Sex f/m	13/14	14/13	Fisher's=1.0
Ethnicity Caucasian/African-Caribbean/ se Asian/ African/ Asian/ other	15/2/1/1/2/6	12/7/2/2/3/1	Chi sq=0.426
Social class (parent's occupation) ab,c1,c2,de, unknown	2/6/9/3/7	2/14/6/1/4	Chi sq=0.23
National Adult Reading Test mean(SD)	28.69(12.24)	32.89(7.96)	P=0.147
Current antipsychotic or antidepressant medication y/n	6/21	0/27	Fisher's=0.023
Education no qualifications/ GCSE/A-level/ degree/higher degree	2/13/5/7/0	0/3/16/6/2	Chi sq=0.003
Tobacco (ever used) y/n	19/8	7/20	Fisher's=0.002
Alcohol (ever used) y/n	22/5	22/5	Fisher's=1
Cannabis (ever used) y/n	19/8	14/13	Fisher's=0.264
Cannabis - times taken in previous year (SD)	13.04(44.5)	67.11(223.8)	P=0.228

Table 2: clinical measures

	ARMS (n=27) Mean(SD)	Controls (n=27) Mean(SD)	Significance (p)
CAARMS –abnormalities of thought content	3.3(1.56)	0.15(0.456)	<0.001
CAARMS –perceptual abnormalities	2.37(1.75)	0.37(0.967)	<0.001
CAARMS –speech abnormalities	1.00(1.441)	0.07(0.385)	0.002
PANSS – Positive	11.67(3.328)	7.26(0.813)	<0.001
PANSS – Negative	8.93(3.05)	7.19(0.786)	0.007
PANSS – General	22.2(4.46)	16.8(1.18)	<0.001
HAM-A	12.52(11.37)	1.85(2.63)	<0.001
HAM-D	10.52(8.85)	1.63(2.71)	<0.001

Table 3: metabolite measures (mean(SD)) in anterior cingulate, left thalamus and left hippocampus (* $p<0.05$).

	Anterior Cingulate		Left Hippocampus		Left Thalamus	
	Controls	ARMS	Controls	ARMS	Controls	ARMS
Glu	11.93(2.01) n=27	12.7(3.14) n=26	7.31(1.65) n=27	6.94(1.54) n=24	7.62(1.32) n=26	6.76(1.18) n=26*
Gln	6.57(1.44) n=12	10.01(3.49) n=9*	5.13(0.82) n=4	5.72(1.03) n=5	5.52(0.69) n=2	4.10(NA) n=1
Glu+Gln	16.63(3.12) n=27	18.28(6.15) n=27	9.91(2.52) n=27	10.27(2.91) n=24	9.75(2.39) n=25	8.37(1.73) n=23*
NAA	10.25(1.14) n=27	11.15(2.27) n=27	7.79(1.08) n=27	7.79(1.57) n=26	11.59(0.89) n=27	10.82(0.89) n=27*
GPC (Cho)	2.56(0.64) n=26	2.6(0.6) n=24	1.91(0.35) n=24	1.91(0.58) n=21	2.02(0.19) n=21	1.91(0.28) n=23
mI	7.65(0.93) n=27	7.91(1.93) n=27	5.88(1.33) n=27	6.11(3.2) n=25	4.14(0.65) n=27	3.99(0.71) n=26
Cre	9.19(1.33) n=27	9.6(2.01) n=27	6.27(1.07) n=27	6.39(1.82) n=26	7.06(0.71) n=27	6.58(0.55) n=27*

Figure 1: differences in gray matter volume between ARMS and controls (n=27). The ARMS group had significantly lower cortical gray matter volumes in orbitofrontal cortex bilaterally, extending into the adjacent ventral anterior cingulate cortex, but relatively more gray matter volume in left cerebellum and left occipital cortex

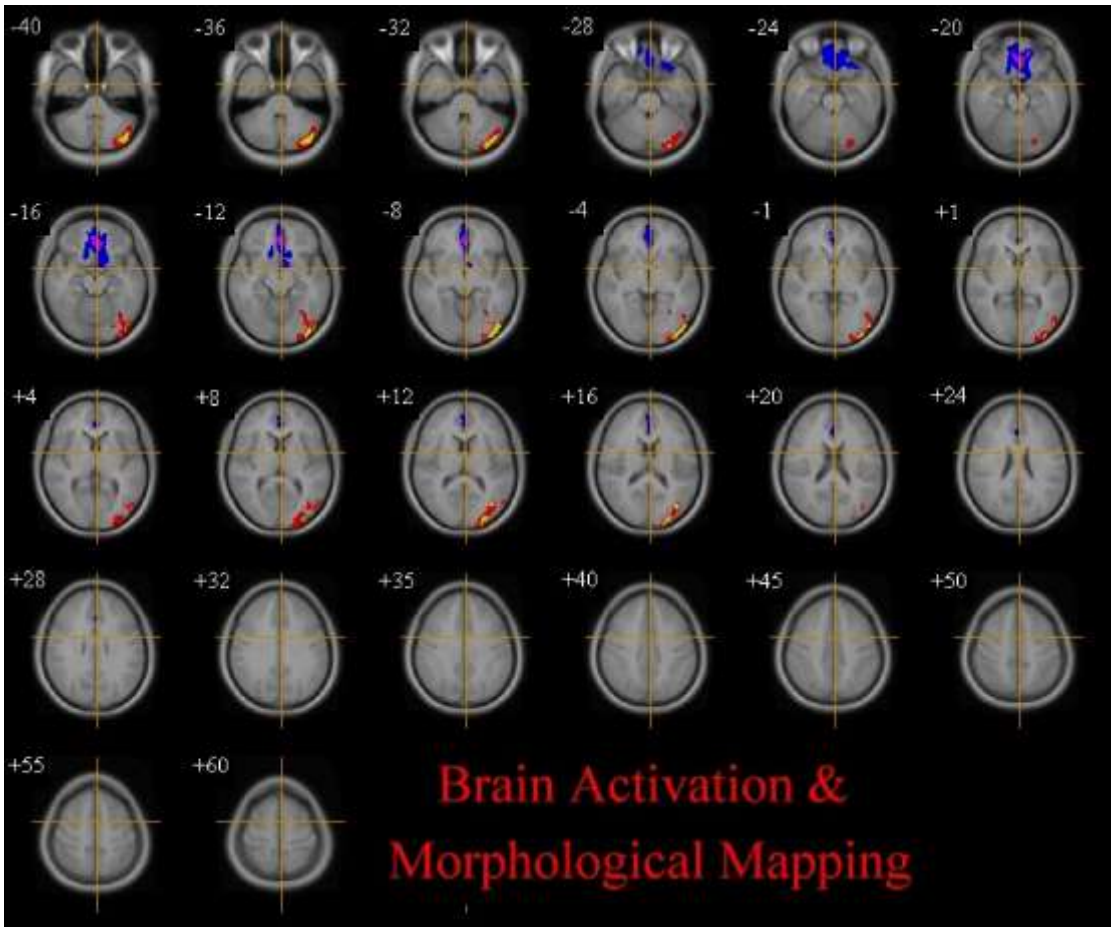
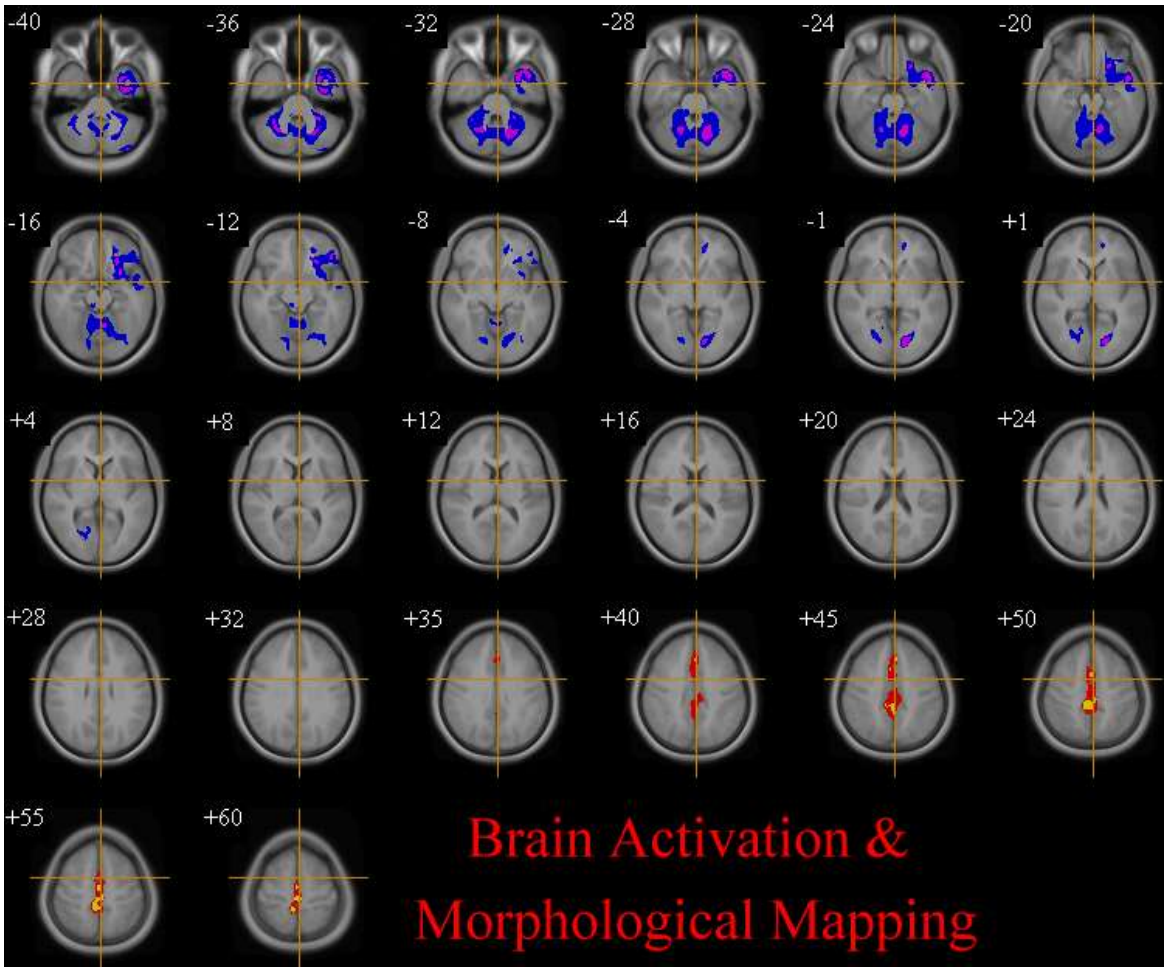


Figure 2: significant correlations between thalamic glutamate and gray matter volume in ARMS subjects (n=26). Thalamic glutamate levels correlated directly with lower gray matter volume in left prefrontal cortex, insula, cingulate, superior temporal gyrus and temporal pole, as well as bilaterally in the cerebellum and lingual gyrus. They showed an inverse correlation with gray matter volume in dorsal anterior cingulate extending to the posterior cingulate gyrus.



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